The dependence of the various kinds of microbial metabolism on the redox state of the medium

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I. INTRODUCTION

From the point of view of microbial life, one of the most salient physico-chemical features of the recent sedimentary environment is the occurrence of a steep vertical gradient of oxidation-reduction conditions.

This gradient is caused by organic material within the sediment but it, in turn, influences the kind of microbiological metabolism likely to occur.

The characterization of the redox conditions and the knowledge of the relationship between redox conditions and microbial metabolism is therefore very important for an understanding of the biological processes within the sediments.

II. SIGNIFICANCE OF THE REDOX POTENTIAL CONCEPT AND MEASUREMENT IN NATURAL ENVIRONMENTS

In a medium where all the redox couples Red_i/Ox_i, reacting according to the equation,

$$Red_i + m_i\ H_2O \rightleftarrows Ox_i + 1_i\ H^+ + n_i\ e^-,$$

are in thermodynamic equilibrium each with respect to the other, the redox potential (E_h) of this medium is given by the Nernst relation :

$$E_h = \frac{\Delta G^\circ i}{n_i F} + \frac{RT}{n_i F} \ln \frac{a(Ox_i)}{a(Red_i)} - \frac{RT1_i}{n_i F}\ pH \qquad \text{(for all i's).}$$

For obvious practical reasons, this theoretical definition can be extended to the cases of media in metastable or partial equilibria. In such cases, the E_h is defined with respect to the only couples which are reactive enough in the considered conditions to reach mutual equilibrium. (The term "conditions" includes the presence or absence of specific catalysts, such as micro-organisms).

Most natural media are dynamic systems subject to an energy flow. This energy flow is fed either by sunlight, used by photoautotrophs to syntesize highly unstable organic molecules, or by a direct input of these organic molecules from outside or from another part of the ecosystem. Heterotrophic and chemoautotrophic metabolisms dissipate this energy flow by tending to bring the system back to its thermodynamic equilibrium. The stationary state eventually reached in such dynamic systems cannot a priori be described by a thermodynamic equilibrium model, a n d the significance of the concept of redox potential in natural environments is therefore questionable.

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However, many observations concerning the distribution of chemical species in natural media displaying gradients of redox conditions and subjected to intense bacterial activity (Richards, 1965; Thorstenson, 1970; Billen 1975; Billen et al., 1976) show that an internal thermodynamic equilibrium is not far from being attained in the sub-systems formed by the main mineral redox couples involved in the energy metabolism of micro-organisms - such as H_2O/O_2 or H_2O_2/O_2 (Sato, 1960; Breck, 1972), Mn^{2+}/MnO_2, NH_4^+/NO_3^-, $Fe(OH)_3/FeCO_3$, SO_4^{2-}/HS^-, NH_4^+/N_2, CH_4/CO_2 (Fig. 1).

The redox potential of natural environments can therefore be best defined with respect to this sub-system, considered, as a first approximation - be be at equilibrium. So defined, it does not take into account the presence of highly reduced organic matter, but only characterizes the availability of oxidants susceptible to use for the energy metabolism of micro-organisms.

By definition, the best way to determine the redox potential is to measure the concentration of the two redox forms of a particular couple Red_i/Ox_i. Thus, some couples which occur in most environments can be used as natural redox indicators e.g. Mn^{2+}/MnO_2; $Fe^{2+}/Fe(OH)_3$. The direct measurementof E_h by the traditional platinum electrode, although it can provide valuable relative indications, must be interpretated with caution, amongst other reasons because of the different reactivity of some redox substances at the platinum electrode and in solution.

III. OCCURRENCE OF VARIOUS REDOX MICROBIAL METABOLISMS IN NATURAL ENVIRONMENTS

Fermentative metabolisms result in reoganization of organic matter without modifying their gross redox level (see footnote). Therefore, they remain insensitive to the redox conditions of the medium. On the other hand, the respiratory metabolism, either organo- or chemo-lithotrophic, consists of the oxidation of a reduced substrate by an oxidant present in the medium. Not every combination of donor substrate with final acceptor is possible in all environments since the electron transfers have to be exoenergetic for its use as an energy-yielding metabolic system.

This allows us to define the general and ecological conditions for the existence of various respiratory metabolisms.

A necessary condition for the existence of a metabolic system is that the mean free energy change per electron during the oxidation of a donor couple by the acceptor couple must be negative under standard conditions (25°C, 1 atm. pressure, unit activity of all chemical species except at $aH^+ = aOH^- = 10^{-7}$). This free energy change can be directly calculated from a comparison of the standard redox potential at pH 7 of the couples concerned. Such a comparison is represented in Fig. 2 according to a scheme used by MacCarthy (1972). In this representation, the mean free energy change per electron is the intersection of the horizontal line, corresponding to the acceptor couple, with the oblique line, corresponding to the donor substrate. There are few metabolisms predicted possibly by this figure which do not occur in natural environments (no bacterium has as yet been isolated which oxidizes NH_4^+ to N_2, or N_2 to NO_3^- as an energy-yielding process, although these would be thermodynamically conceivable).

Note: An exception to this is when molecular hydrogen is produced during the fermentation. This case presents some - more than a formal - analogy with respiratory metabolisms, of which it could represent a primitive form (Gray and Gest, 1965).

In a particular environment, the free energy change associated with the oxidation of a reduced substrate is a function of the availability of an oxidant, and thus of the E_h of this environment as we have so defined it. Considering a natural medium in which the sub-system formed by the mineral redox couples is at thermodynamic equilibrium at a certain value of pH and E_h :

$$E_h = E_i^{!\circ} + \frac{RT}{n_i F} \ln \frac{Ox_i}{Red_i} .$$

If a reduced substrate, S_{red}, organic or mineral, is brought into this medium, which is considered to be of infinite size, irrespective of which oxidant used, the maximal energy provided by the oxidation of the substrate will be given by :

$$\Delta G = nF\, E'^{\circ}_{Sred/Sox} + RT \ln Sox/Sred - nF\, E_h .$$

This relationship is graphically represented for some substrates in Fig. 3. From this it can be concluded that, within the range of E_h encountered in natural media (Baas Becking et al., 1960), oxidation of organic compounds (i.e. chemo-organotrophic metabolisms) is always exoenergetic, although the energy yield is lower at lower E_h.

For chemolithotrophic metabolisms on the other hand, stricter limitations exist since the oxidation of mineral substrates is not exoenergetic over the whole E_h range. Thus it can be predicted that Thiobacter are active only at pH and E_h values higher than the upper limit of stability of the reduced sulphur compounds they metabolize. Observations by Baas Becking and Wood in 1955 entirely confirm this prediction (Fig. 4). Likewise, ferro-oxidizing bacteria are only active above the stability limit of iron carbonate, as shown by the data compiled by Baas Becking et al. (1960) in Fig. 5. Nitrification occurs only at E_h values at which oxidation of ammonium into nitrate is exoenergetic, as shown both by observations on pure cultures by Zobell (1935) and by direct measurements of nitrifying activity in coastal marine sediments and in the Scheldt estuary by Billen (1976) and Somville (unpublished).

The preceding thermodynamic considerations only concern the possibility of reduced substrate oxidation not allowing the prediction, as a function of E_h, of which oxidant may be employed in a respiratory metabolism. However, many observations suggest that various oxidants are used successively, in order of their decreasing E°'; aerobic respiration will occur first and thus denitrification will not occur in the presence of oxygen or a E_h values higher than + 250 mV (Fig. 6); sulphate-reduction is inhibited by oxygen (Wheatland, 1954) and by nitrate (Fig. 7); methane production begins only after sulphates are completely exhausted (Martens and Berner, 1974). Although direct experimental data are lacking concerning these metabolisms, by analogy, it can be predicted that utilization of Mn(IV) and Fe(III) as oxidants will occur respectively after aerobic respiration and after denitrification.

Although this sequence of utilization allows the best energy yield to be obtained from the oxidation of reduced substrates, no thermodynamic argument fully explains it. One explanation could involve the existence of a competition between micro-organisms for reduced substrates, with selection of the energetically more favourable metabolic system. However, such a reason could not explain the conditions in environments rich in organic deposits where reduced substrates are not limiting. Direct physiological effects do exist to prevent the use of one oxidant in the presence of a better one. These physiological effects include :

(i) A general, non-specific toxicity of some oxidants for oganisms not adapted to them. This is the case with oxygen in obligate anaerobic bacteria. Such an

effect is, however, unlikely for poorly reactive oxidants such as nitrate or sulphate.

(ii) The specific action of an oxidant on the electron transport system of micro-organisms normally adapted to use another one, e.g. by "drainage" of the electrons towards the better oxidant; and

(iii) The regulation of the synthesis and activity of enzymes involved in redox metabolism. When an organism is adapted to use several energy-yielding metabolisms, regulatory mechanisms generally exist which allow the optimal one to be used for a given set of environmental conditions (e.g. the switch from fermentation to respiration when oxidants become available or from denitrification to aerobic respiration when the oxygen concentration rises).

IV. CONCLUSION

The redox potential in natural systems, defined with respect to the sub-system formed by the main mineral couples involved in the energy-yielding metabolism of micro-organisms, is an important parametre from which the type of metabolism which will occur under a particular set of conditions can be deduced. In diagenetic models of recent sediments, it can therefore be a useful conceptual - as well as empirical - parametre for introducing vertical zoning with different types of microbiological activities occurring successively with increasing depth.

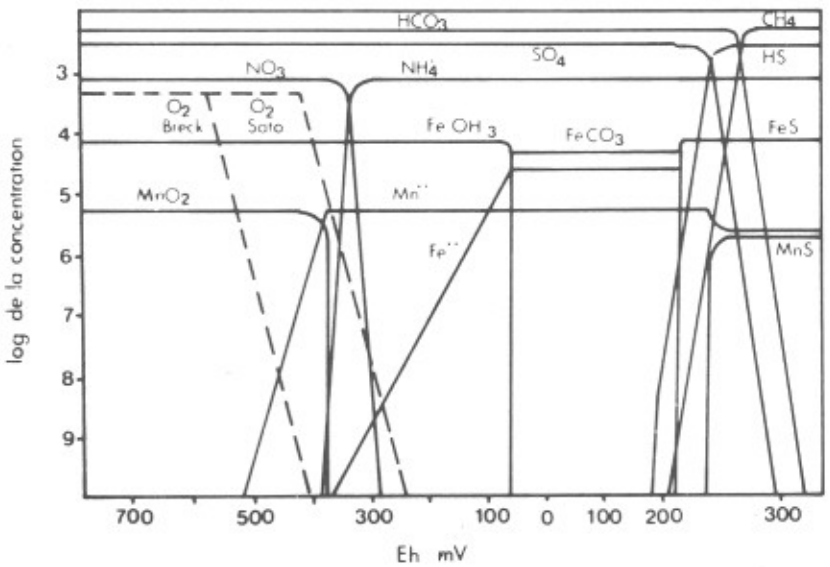

Fig. 1. Equilibrium diagram of the system H_2O_2/O_2, Mn^{2+}/MnO_2, NH_4^+/NO_3^- $Fe(OH)_3/FeCO_3$, SO_4^{2-}/HS^-, CH_4/CO_2, in a typical freshwater at pH 7.5.

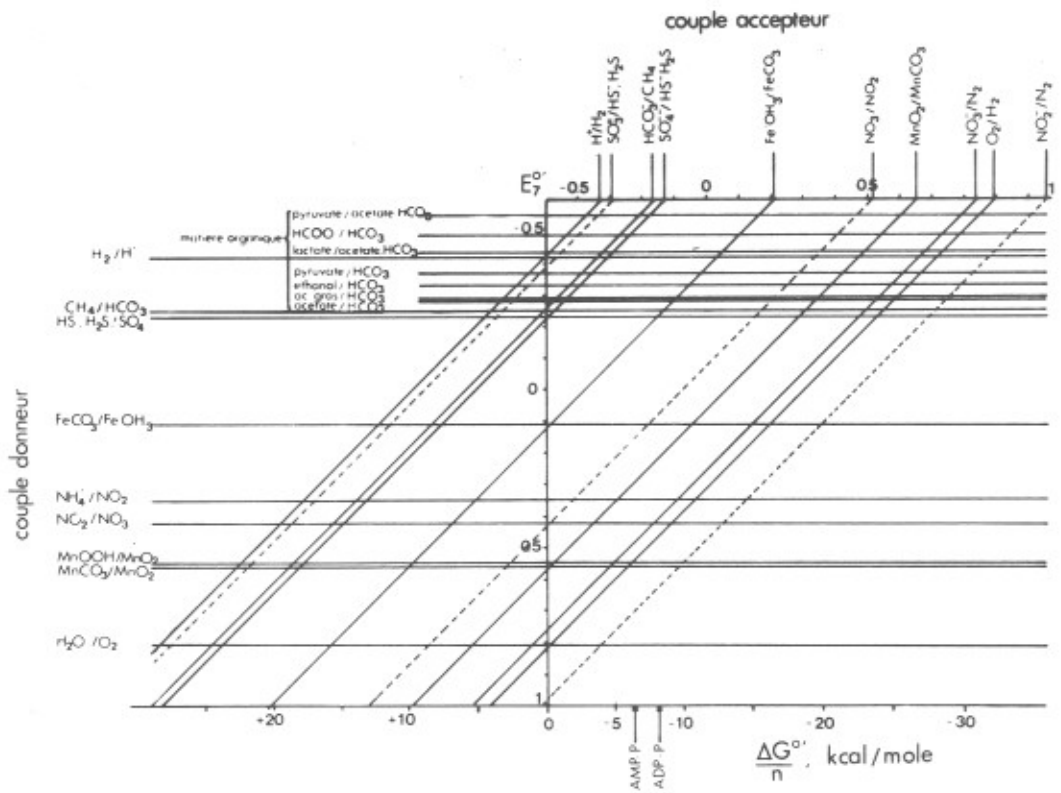

Fig. 2 Free energy change per electron during the oxidation of a donor couple at STP; (thermodynamic data from Garrels and Christ, (1965) and Edsall and Wymann (1958).

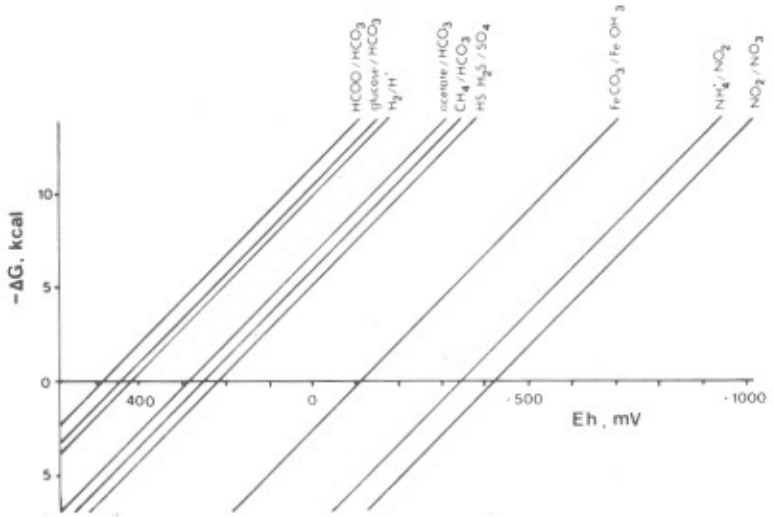

Fig. 3 Maximum free energy change per electron during some biological oxidations (organotrophic and lithotrophic metabolisms) as a function of the redox potential of the environment (at pH 7).

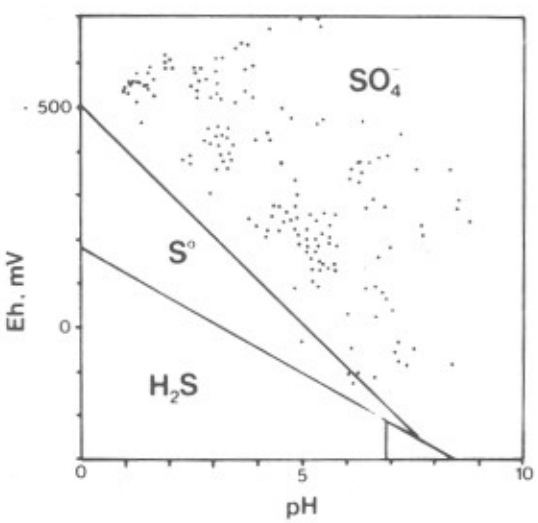

Fig. 4 E_h and pH in active cultures of Thiobacillus on sulphur (from Baas Becking and Wood, 1955). Thermodynamic stability of sulphur with respect to sulphates.

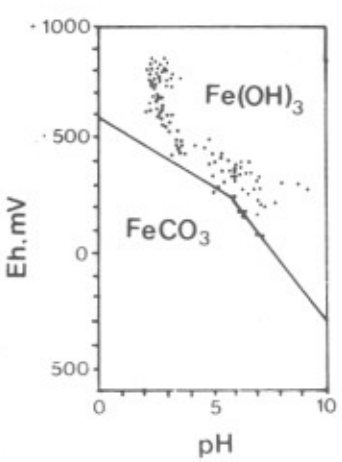

Fig. 5 Zone of activity of ferro-oxidizing bacteria in pure cultures and in natural environments (from Baas Becking et al., 1960). Thermodynamic stability zone of ferric hydroxide and ferrous carbonate.

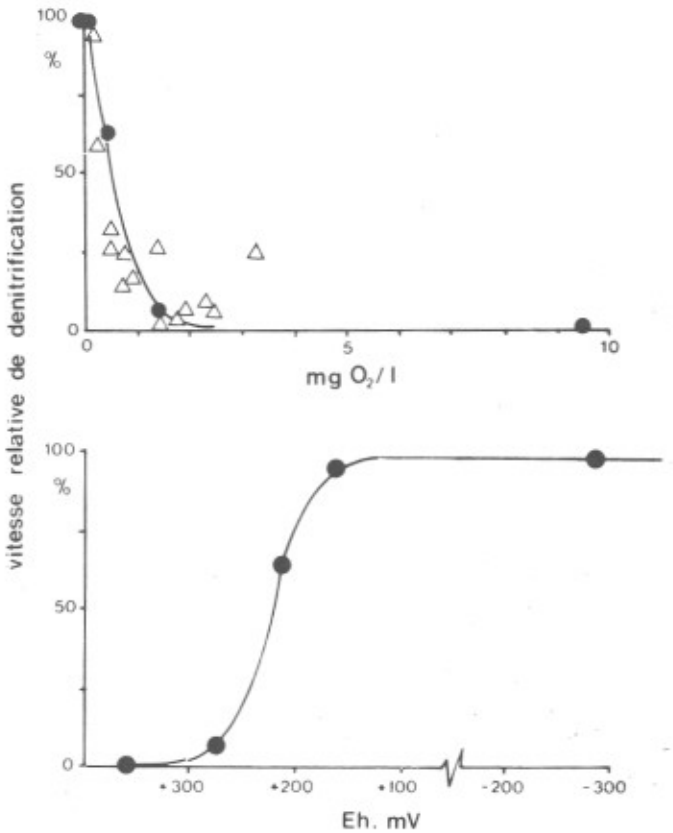

Fig. 6 Effect of oxygen concentration and E_h on denitrification. (□) Data of Wheatland (1959).
(●) Experiment on a suspension of muddy sediments from the Sluice Dock at Ostend (Belgium).

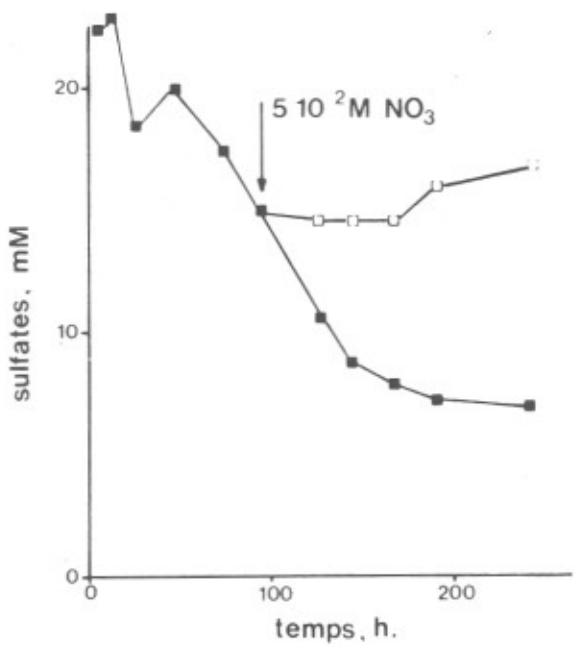

Fig. 7 Effect of the addition of nitrate on sulphate reduction in a suspension of muddy sediment from the Sluice Dock at Ostend.

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